

## PRODUCTION OF SELECTED BACULOVIRUSES IN NEWLY ESTABLISHED LEPIDOPTERAN CELL LINES

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(Received 27 December 2000; accepted 16 March 2001)

### SUMMARY

One key to the in vitro mass production of baculoviruses is the development of insect cell lines capable of producing high levels of extracellular virus (ECV) and/or occlusion bodies (OBs). For this study, 34 newly established cell lines from 10 lepidopteran species were screened for their ability to produce ECV and OBs from a variety of baculoviruses. The selected baculoviruses included: the alfalfa looper virus (AcMNPV); the celery looper virus (AfMNPV); the velvetbean caterpillar virus (AgMNPV), the bollworm virus (HzSNPV), the diamondback moth virus (PxMNPV), and the beet armyworm virus (SeMNPV). ECV titers were determined using TCID<sub>50</sub> assays (50% tissue culture infectivity dose), with the presence or absence of OBs being noted. For AcMNPV, 28 new cell lines were tested, with eight producing AcMNPV ECV titers of  $1.1\text{--}47.3 \times 10^6$  TCID<sub>50</sub>/ml and 11 producing OBs. For AgMNPV, six new cell lines were tested, with all producing AgMNPV ECV titers of  $3.5\text{--}62.3 \times 10^6$  TCID<sub>50</sub>/ml and generating OBs. For HzSNPV, four new cell lines were tested with three lines producing HzSNPV ECV titers of  $1.4\text{--}5.0 \times 10^6$  TCID<sub>50</sub>/ml, but none generating OBs. For PxMNPV, 10 new cell lines were tested with seven generating PxMNPV ECV titers of  $4.7\text{--}232.6 \times 10^6$  TCID<sub>50</sub>/ml and eight producing OBs. Lastly, using qualitative or semiquantitative methods, homologous cell lines were tested for AfMNPV and SeMNPV production, all of which produced OBs. Overall, many of the cell lines tested were found to produce OBs and generate moderate to high levels of ECVs of one or more baculoviruses.

**Key words:** insect; AcMNPV; AfMNPV; AgMNPV; HzSNPV; PxMNPV; SeMNPV.

### INTRODUCTION

Baculoviruses are known to effectively control agronomically important pest insects in the field (Moscardi, 1989; Agathos, 1991; Murhammer, 1996; Black et al., 1997). These biopesticides can be produced either in insect larvae or in insect cell lines (Weiss and Vaughn, 1986; Agathos, 1991; Weiss et al., 1992; Goodman and McIntosh, 1994; Murhammer, 1996; Black et al., 1997), but optimization is needed for their production in cell culture. Numerous factors are important for the mass production of baculoviruses by insect cell lines, including the development of high virus-producing cell lines, low-cost serum-free media, and methods for deterring mutant virus formation (Fraser and Hink, 1982; Weiss and Vaughn, 1986; Agathos, 1991; Weiss et al., 1992; Goodman and McIntosh,

1994; Murhammer, 1996; Black et al., 1997). The main objective of the present study was to screen newly established lepidopteran cell lines for their ability to produce selected baculoviruses.

The baculoviruses selected for production included a singly enveloped virus from *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (HzSNPV) and five multiply enveloped viruses from *Autographa californica* (Speyer) (Lepidoptera: Noctuidae) (AcMNPV), *Anagrapha falcifera* (Kirby) (Lepidoptera: Noctuidae) (AfMNPV), *Anticarsia gemmatilis* (Hübner) (Lepidoptera: Noctuidae) (AgMNPV), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (PxMNPV), and *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) (SeMNPV). These viruses vary in their levels of specificity: AcMNPV and AfMNPV are infectious to numerous lepidopteran species, including many of the species from which we obtained new cell cultures, such as (for one or both viruses) *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae), *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae), *P. xylostella*, *S. exigua*, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), and *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae); AgMNPV is known to have a narrow host range which includes *A. gemmatilis*, *H. virescens*, and *T. ni*; HzSNPV is specific for heliothine species, including *H. virescens* and *H. zea*; PxMNPV has a broad host range similar to AcMNPV; and SeMNPV has a narrow host range which includes its homologous host (Moscardi, 1989; McIntosh and Grasele, 1994; Castro et al., 1997; Kariuki and McIntosh, 1999).

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<sup>3</sup> Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

In the present study, the selection of cell lines for the production of the various baculoviruses was based partially on their *in vivo* specificities. Numerous studies have indicated that the ability of baculoviruses to replicate in cell lines is often related to the species of origin of the cell line and the ability of the virus to infect insects of that same species (as reviewed by McIntosh and Grasela, 1994). These studies have also indicated that a baculovirus may infect a certain insect species but not a specific cell line derived from this insect, which may be due to the tissue of origin of the cell line. Furthermore, *in vivo* studies have indicated that some baculoviruses replicate preferentially in specific insect tissues such as fat body (Vail and Jay, 1973). In contrast, studies involving cell lines from different tissues have not established a definite relationship between the tissue of origin and the virus replication ability, especially if different viral strains or species are used when testing (as reviewed by Goodman and McIntosh, 1994). Therefore, a second objective of this study was to compare the production levels of different viruses in cell lines from a variety of insect species and tissue sources.

#### MATERIALS AND METHODS

**Cell lines.** Newly established cell lines from numerous lepidopteran species and tissues were previously generated in BCIRL (Grasela et al., 2000; Goodman et al., 2001; Table 1). Four previously established cell lines were used for comparison: Sf9, BCIRL-HvAM1 (McIntosh et al., 1981), BCIRL-HzAM1 (McIntosh et al., 1985), and TnCL1 (a clone of TN-368; McIntosh and Rechteris, 1974). Cell lines, excluding HvAM1, were maintained in ExCell 401<sup>®</sup> medium (JRH Biosciences,<sup>3</sup> Lenexa, KS), either with or without 10% fetal bovine serum (FBS) (Intergen, Purchase, NY). HvAM1 was maintained in TC-199MK with 10% FBS (McIntosh et al., 1973). All culture media contained 50 U/ml penicillin and 0.05 mg/ml streptomycin (Sigma Chemical Co., St. Louis, MO).

**Baculovirus production.** Viruses tested in the production studies were: AcMNPV, clone V8 (developed by American Cyanamid Co., Princeton, NJ; produced in Sf9 cells using ExCell 401); AfMNPV, clone 1 (McIntosh, 1991; produced in TnCL1 cells using ExCell 401); AgMNPV, clone 4-3A1 (Grasela and McIntosh, 1998; produced in HvAM1 cells using ExCell 401 + 10% FBS); HzSNPV, Brownsville strain, clone 2 (Lenz et al., 1991; produced in HzAM1 cells using ExCell 401); PxMNPV, clone 3 (Kariuki and McIntosh, 1999; produced in TnCL1 cells using ExCell 401); and SeMNPV, a wild-type strain originally described by Kolodny-Hirsch et al. (1993) (obtained from Du Pont, Boston, MA; produced in BCIRL/AMCY-SeE4 cells using ExCell 401). Virus production was performed in either serum-free or serum-containing medium, as indicated. In some cases, cell lines were tested in both media. Cells ( $2 \times 10^5$  cells/ml) were inoculated with 1 MOI (multiplicity-of-infection) of extracellular virus (ECV), and rocked for a minimum of 2 h. The inoculum was then removed, and the cells were washed twice with Hanks' balanced salt solution (50 U/ml penicillin, 0.05 mg/ml streptomycin), and fresh medium was added. The cells were incubated at 28°C for 5–7 d, after which the virus was harvested using low-speed centrifugation ( $1000 \times g$ , 10 min). ECV titers were determined by dilution end-point titrations (50% tissue culture infectivity dose [TCID<sub>50</sub>]), with the following cell lines ( $10^4$  cells/well, 0.2 ml/well) being used as indicator lines (with media indicated): HvAM1 for AcMNPV (TC199-MK) and AgMNPV (ExCell 401 + 10% FBS); HzAM1 for HzSNPV (ExCell 401); TnCL1 for PxMNPV (ExCell 401); and SeE4 for SeMNPV (ExCell 401 + 10% FBS). The percentage of the inoculated cells that either produced occlusion bodies (OBs) or showed cytopathological effects, such as granular formations, was determined by visualization using an IM 35 inverted microscope (Carl Zeiss, Thornwood, NY). Kruskal-Wallis one-way analysis of variance on ranks was used to compare whole datasets for a given virus treatment, and two-sample *t*-tests were performed for pair-wise comparisons of selected means within those sets (SigmaStat, Jandel Scientific, Chicago, IL; NCSS, Kaysville, UT).

#### RESULTS

ECV titers and percentage of cells infected and/or exhibiting OBs were determined quantitatively in a variety of lepidopteran cell lines

inoculated with selected baculoviruses. Since only two replications were made per sample, statistically significant differences were often not seen for values when data from all the cell lines for one virus were compared, although pair-wise comparisons of selected lines in some cases yielded significant differences. AcMNPV production was evaluated in 28 new cell lines and three previously established lines (Sf9, HvAM1, HzAM1) (Table 2). Eight new lines produced ECV titers higher than  $1 \times 10^6$  TCID<sub>50</sub>/ml, and 11 lines generated OBs. ECV titers of all the cell lines were statistically compared ( $P = 0.05$ ) with those of Sf9 (a cell line widely used for AcMNPV production; Agathos, 1991). HvE1, HvOV, and HzE6 produced statistically similar titers of ECV when compared with Sf9, while SftS produced significantly higher titers of ECV than those produced by Sf9, with all other lines producing significantly lower titers. For AgMNPV production, five out of the six new lines tested generated high virus titers (i.e.,  $\geq 10^7$  TCID<sub>50</sub>/ml), and most of these cell lines had  $\geq 70\%$  of their cells producing OBs (Table 3), with no statistically significant differences. Selected *H. zea* lines were tested for HzSNPV replication in ExCell 401 containing 10% FBS, resulting in low to moderately high ECV titers and no OBs being generated. HzSNPV ECV titers for these lines were (TCID<sub>50</sub>/ml,  $\pm$  standard error of the mean [SEM]):  $2.38 \pm 0.23 \times 10^6$  (HzE1, P37);  $5.04 \pm 1.69 \times 10^6$  (HzE2, P41);  $0.02 \pm 0.005 \times 10^6$  (HzE3, P12);  $1.41 \pm 0.52 \times 10^6$  (HzE8, P12); and  $8.41 \pm 0.87 \times 10^6$  (for HzAM1, P94; a cell line known to be a moderate producer of HzSNPV; Lenz et al., 1991). Ten new cell lines were examined for PxMNPV production (Table 4). Four of these new lines produced high virus titers ( $\geq 1 \times 10^7$  TCID<sub>50</sub>/ml), and most generated OBs, with SftS being the cell line exhibiting the highest percentage of cells producing OBs. Statistically significant differences for PxMNPV ECV production were not observed between TnCL1 (a cell line reported to be a high producer of PxMNPV; Kariuki et al., 2000) and HvOV ( $\pm$  FBS), SftS, and HvE2 (with FBS) ( $P = 0.05$ ), with all the other cell lines producing lower ECV titers.

Additional qualitative or semiquantitative studies were performed on the replication of AfMNPV and SeMNPV by their homologous cell lines, AfoV for AfMNPV and the lines SeE1, SeE4, and SeE5 for SeMNPV. AfoV was found to produce AfMNPV OBs readily in serum-free ExCell 401 medium, although ECV titers were not determined. For SeMNPV, the three SeE lines produced  $1\text{--}7 \times 10^6$  TCID<sub>50</sub>/ml in serum-free ExCell 401 and  $8\text{--}14 \times 10^6$  TCID<sub>50</sub>/ml in ExCell 401 containing 10% FBS, with OBs being generated in all the cases.

#### DISCUSSION

Newly established cell lines from a variety of insect species and tissue sources produced moderate to high levels of ECV of selected baculoviruses and, in some cases, OBs. Interestingly, not all the cell lines which produced appreciable levels of ECV were able to generate OBs (e.g., AcMNPV production by HzE6; Table 2). Furthermore, this latter example is the first observation of AcMNPV production by an *H. zea* cell line and indicates that a cell line's ability to produce ECV of a selected virus species is not always related to the *in vivo* species specificity of the given virus, although this relationship has generally held true (as reviewed by McIntosh and Grasela, 1994). Similarly, Kariuki et al. (2000) found that an *H. virescens* (HvAM1) and a *T. ni* (TnCL1) cell line were better producers of PxMNPV than the homologous embryonic cell line

TABLE 1

CELL LINES USED FOR VIRUS PRODUCTION<sup>a</sup>

Abbreviated designation	Complete designation	Species of origin	Tissue of origin
AfOV	BCIRL/AMCY-AfOV-CLG	<i>Anagrapha falcifera</i>	Adult ovarian/fat body
AgOV1	BCIRL/AMCY-AgOV-CLG1	<i>Anticarsia gemmatalis</i>	Adult ovaries/fat body
AgOV2	BCIRL/AMCY-AgOV-CLG2	<i>A. gemmatalis</i>	Adult ovaries/fat body
AgOV3	BCIRL/AMCY-AgOV-CLG3	<i>A. gemmatalis</i>	Adult ovaries/fat body
AgE	BCIRL/AMCY-AgE-CLG	<i>A. gemmatalis</i>	Embryonic
AiOV	BCIRL/AMCY-AiOV-CLG	<i>Agrotis ipsilon</i>	Adult ovaries/fat body
AiTS	BCIRL/AMCY-AiTS-CLG	<i>A. ipsilon</i>	Adult testes/fat body
HvE1	BCIRL/AMCY-HvE-CLG1	<i>Heliothis virescens</i>	Embryonic
HvE2	BCIRL/AMCY-HvE-CLG2	<i>H. virescens</i>	Embryonic
HvE3	BCIRL/AMCY-HvE-CLG3	<i>H. virescens</i>	Embryonic
HvTS	BCIRL/AMCY-HvTS-GES	<i>H. virescens</i>	Larval testes
HvOV	BCIRL/AMCY-HvOV-CLG	<i>H. virescens</i>	Adult ovaries
HZE1	BCIRL/AMCY-HZE-CLG1	<i>Helicoverpa zea</i>	Embryonic
HZE2	BCIRL/AMCY-HZE-CLG2	<i>H. zea</i>	Embryonic
HZE3	BCIRL/AMCY-HZE-CLG3	<i>H. zea</i>	Embryonic
HZE5	BCIRL/AMCY-HZE-CLG5	<i>H. zea</i>	Embryonic
HZE6	BCIRL/AMCY-HZE-CLG6	<i>H. zea</i>	Embryonic
HZE7	BCIRL/AMCY-HZE-CLG7	<i>H. zea</i>	Embryonic
HZE8	BCIRL/AMCY-HZE-CLG8	<i>H. zea</i>	Embryonic
OnFB1	BCIRL/AMCY-OnFB-GES1	<i>Ostrinia nubilalis</i>	Larval fat body
OnFB3	BCIRL/AMCY-OnFB-GES3	<i>O. nubilalis</i>	Larval fat body
PxE	BCIRL/AMCY-PxE-CLG	<i>Plutella xylostella</i>	Embryonic
PxLP	BCIRL/AMCY-PxLP-CLG	<i>P. xylostella</i>	Homogenized larva/pupae
SeE1	BCIRL/AMCY-SeE-CLG1	<i>Spodoptera exigua</i>	Embryonic
SeE4	BCIRL/AMCY-SeE-CLG4	<i>S. exigua</i>	Embryonic
SeE5	BCIRL/AMCY-SeE-CLG5	<i>S. exigua</i>	Embryonic
SfTS	BCIRL/AMCY-SfTS-GES	<i>Spodoptera frugiperda</i>	Larval testes
TnE1	BCIRL/AMCY-TnE-CLG1	<i>Trichoplusia ni</i>	Embryonic
TnE1MK	BCIRL/AMCY-TnE-CLG1MK	<i>T. ni</i>	Embryonic
TnE2	BCIRL/AMCY-TnE-CLG2	<i>T. ni</i>	Embryonic
TnE2MK	BCIRL/AMCY-TnE-CLG2MK	<i>T. ni</i>	Embryonic
TnE3	BCIRL/AMCY-TnE-CLG3	<i>T. ni</i>	Embryonic
TnTS1	BCIRL/AMCY-TnTS-GES1	<i>T. ni</i>	Larval testes
TnTS3	BCIRL/AMCY-TnTS-GES3	<i>T. ni</i>	Larval testes

<sup>a</sup> The establishment of these cell lines is described in Grasela et al. (2000) and Goodman et al. (2001). Designations include (in order of appearance): initials of the laboratory (BCIRL, Biological Control of Insects Research Laboratory), corporate collaborator (AMCY, American Cyanamid Co.), insect species (as indicated in italics in the Species column), insect stage or tissue type (as indicated in the Stage or Tissue column), cell line initiator (CLG or GES, see author list), and cell line number (if more than one of the specified type exists).

PxEM1, although their earlier studies had indicated that *P. xylostella* larvae are more susceptible to infection by PxMNPV than *H. virescens* or *T. ni* larvae (Kariuki and McIntosh, 1999). Likewise, in our studies, we observed that the *P. xylostella* cell lines generated lower PxMNPV ECV titers than many of the *H. virescens* and *T. ni* lines (Table 4). Our ability to compare the virus production in cell lines from different tissues of the same species was limited by the sample numbers used, although statistically significant differences were observed in a few cases. For example, the ovarian cell line from *H. virescens* produced significantly higher AcMNPV ECV titers than the *H. virescens* line from larval testes, although this trend was not observed for the *S. frugiperda* ovarian (Sf9) and larval testes (SfTS) lines (Table 2). Additionally, significant differences in AcMNPV ECV titers were observed between cell lines from the embryonic tissues of either *H. virescens* or *H. zea* (approximately 10-fold differences in both cases) (Table 2). To summarize, we found that neither the species nor the tissue of origin of cell lines may be absolute predictors of the ability of a selected cell line to produce baculoviruses.

Comparisons of our newly developed cell lines with other cell

lines indicate that some of our lines produced comparable, if not higher, levels of ECV of various baculoviruses. For AcMNPV clone V8 titers, HvE1, HvOV, HZE6, and SfTS produced similar or higher ECV than Sf9 and HvAM1 (Table 2). Titers of this magnitude or higher for AcMNPV wildtype or clone E2 were also reported for the cell lines BTI-EAA, BCIRL-HsAM1, IPLB-LD64BA, IZF-MB0503, IPLB-Sf21-AE, Tn-368, TnCL1, BTI-Tn4A14/4B/4B31/5B1-4, and IAL-TnD (Lynn and Hink, 1980; Danyluk and Maruniak, 1989; McIntosh and Ignoffo, 1989; McKenna et al., 1998; Kariuki et al., 2000). Concerning AgMNPV, all the cell lines we tested (which originated from either *A. gemmatalis* or *H. virescens*) produced moderate to high levels of AgMNPV ECV and were similar to the titers generated by HvAM1, but higher than those produced by Sf9 (Table 3). Previous reports of infection of HvAM1 by AgMNPV reported higher ECV titers, although these studies were performed in media different from those used in our studies (Grasela and McIntosh, 1998). Another cell line which has been shown to produce AgMNPV ECV titers similar to those produced by our lines is UFL-Ag-286 (Sieburth and Maruniak, 1988; Castro et al., 1997). For HZSNPV replication, we found that the HZE cell lines did not pro-

TABLE 2

PRODUCTION OF AcMNPV BY SELECTED CELL LINES<sup>a</sup>

Cell line	Passage no.	% FBS in ExCell 401 (passages in SFM) <sup>b</sup>	TCID <sub>50</sub> /ml ( $\pm$ SEM)	% Cells infected ( $\pm$ SEM) <sup>c</sup>
Sf9	158	0 (>30)	$16.95 \pm 1.65 \times 10^6$	$76.1 \pm 0.3^d$
HvAM1	261	10 in TC199-MK	$2.35 \pm 0.96 \times 10^6$	$16.1 \pm 2.3$
AfOV	36	0 (11)	$0.29 \pm 0.09 \times 10^6$	$39.5 \pm 6.5$
AgE	52	0 (26)	$0.04 \pm 0.004 \times 10^6$	$35.4 \pm 0.5$
AgOV1	41	0 (26)	$<0.0001 \times 10^6$	$5.2 \pm 1.3$
AgOV2	44	0 (24)	$0.02 \pm 0.00 \times 10^6$	$4.7 \pm 0.6$
AgOV3	59	0 (27)	$0.02 \pm 0.01 \times 10^6$	$59.5 \pm 0.9$
AiOV	62	0 (36)	$0.73 \pm 0.11 \times 10^6$	$8.8 \pm 0.15^{c,d}$
AiT5	36	10	$0.65 \pm 0.21 \times 10^6$	$6.0 \pm 1.9^{d,e}$
HvE1	37	0 (>20)	$8.55 \pm 4.15 \times 10^6$	$39.2 \pm 0.1^c$
HvE2	63	1 (>20 in SFM)	$1.05 \pm 0.10 \times 10^6$	$49.2 \pm 1.2^{d,e}$
HvE3	41	0 (11)	$0.77 \pm 0.18 \times 10^6$	$3.5 \pm 0.9$
HvOV	64	0 (47)	$13.76 \pm 6.65 \times 10^6$	$15.5 \pm 0.6^d$
HvTS	15	10	$0.64 \pm 0.22 \times 10^6$	$17.0 \pm 1.9$
Hze1	30	0 (9)	$0.06 \pm 0.01 \times 10^6$	0.0
Hze2	32	0 (9)	$0.02 \pm 0.01 \times 10^6$	0.0
Hze3	21	0 (7)	$0.28 \pm 0.05 \times 10^6$	0.0
Hze5	19	10	$0.20 \times 10^6$	0.0
Hze6	26	10	$2.17 \pm 0.31 \times 10^6$	0.0
Hze7	16	10	$0.35 \pm 0.24 \times 10^6$	0.0
OnFB1	10	10	$<0.0001 \times 10^6$	$3.8 \pm 0.0$
PxE	49	0 (29)	$0.01 \pm 0.00 \times 10^6$	$2.5 \pm 0.3$
PxLP	60	0 (36)	$0.02 \pm 0.00 \times 10^6$	$0.8 \pm 0.2$
SfTS	40	10	$47.30 \pm 5.10 \times 10^6$	$91.2 \pm 0.8^{d,e}$
TnE1	48	1	$2.62 \pm 1.39 \times 10^6$	$64.8 \pm 2.0^d$
TnE2	43	10	$16.25 \pm 2.35 \times 10^6$	$59.2 \pm 4.1^d$
TnE1MK	34	0 (5)	$0.56 \pm 0.03 \times 10^6$	$29.1 \pm 2.5^d$
TnE2MK	22	10	$0.65 \pm 0.06 \times 10^6$	$64.8 \pm 2.0^d$
TnE3	42	0 (7)	$0.32 \pm 0.02 \times 10^6$	$8.9 \pm 1.9$
TnTS1	19	5 (3)	$2.27 \pm 0.74 \times 10^6$	$37.9 \pm 5.9^{d,e}$

<sup>a</sup> Cells ( $2 \times 10^5$  cells/ml) were inoculated with virus (1 MOI). Virus was harvested 7 d (*H. zea* cell lines) or 5 d (all other cell lines) after inoculation. Values represent mean titer or percentage  $\pm$  SEM ( $n = 2$ ). Designations of cell lines are abbreviated. Complete designations are given in Table 1 and "Materials and Methods" section.

<sup>b</sup> Values in parenthesis indicate the number of passages in SFM.

<sup>c</sup> Percent cells infected denote either cells producing OB or showing CPE such as granular formations.

<sup>d</sup> OB production observed in these cell lines.

<sup>e</sup> Before or on the d of harvest, cell lysis was observed. Therefore, the percent cells showing CPE and/or producing OBs is an underestimation.

duce OBs or high ECV titers, which contrasts with other *H. zea* lines shown to be moderate producers of this virus, such as the parental and clonal cell lines of BCIRL-HzAM1 and IPLB-Hz1075 (Granados et al., 1981; Corsaro and Fraser, 1987; Rice et al., 1989; Lenz et al., 1991). Conversely, replication of PxMNPV by most of the new lines we tested produced similar or higher titers of ECV compared with Sf9 and TnCL1 (Table 4), as well as BCIRL-HzFB3, BCIRL-HsAM1, BCIRL-HvAM1, TnCL1, and Sf21 (Kariuki et al., 2000). Additionally, new cell lines were generated by Lynn and Shapiro (1998) from *H. virescens* embryos and found to replicate numerous baculoviruses, including AcMNPV, AgMNPV, and Hz-SNPV, although ECV titers were not determined. Similarly, some of our cell lines (e.g., HvOV, SfTS, TnE2) were found to be good producers, both of ECVs and OBs, of at least two or three baculoviruses (AcMNPV, AgMNPV, and/or PxMNPV).

Lastly, three characteristics important for the mass production of baculoviruses were exhibited by some of our new cell lines. These characteristics include the ability of the cell lines to: adapt to serum-free media, grow in suspension culture conditions (depending upon the bioreactor employed), and produce stable viruses over several serial passages in cell culture (Weiss and Vaughn, 1986; Aga-

thos, 1991; Weiss et al., 1992; Goodman and McIntosh, 1994; Murhammer, 1996; Black et al., 1997). Previous studies have indicated that some of the newly established cell lines tested in the present study can be adapted to serum-free ExCell 401 medium (viz., AfOV, AgOV1, AgOV2, AgOV3, AgE, AiOV, HvE1, HvE3, HvOV, PxE, PxLP, TnE3, TnE1-MK) and suspension culture in shaker flasks (viz., AiOV, HvE2) (Goodman et al., 2001). Furthermore, preliminary studies on the ability of the SeE1, SeE4, and SeE5 cell lines to produce selected baculoviruses in suspension culture using serum-free medium (SFM) (IPL-10) were performed by Drs. James Vaughn and Cheng-Chung Chou (formerly of Insect BioControl Laboratory, USDA, ARS, Beltsville, MD; pers. comm.). These studies indicated that the SeE1 and SeE4 lines were able to generate OB concentrations ranging from  $0.81$  to  $11.25 \times 10^8$  OBs/ml (or 23–360 OBs/cell) for AcMNPV and SeMNPV. Additional studies by Drs. Vaughn and Martin Shapiro (Insect BioControl Laboratory, USDA, ARS; pers. comm.) concerning the effects of serial passaging of SeMNPV in SeE1 cells using IPL-41 + 10% CPSR3 (six passages) indicated that OB production was elevated, although in vivo OB virulence was decreased.

In conclusion, virus production data indicate that many of our

TABLE 3

PRODUCTION OF AgMNPV BY SELECTED CELL LINES<sup>a</sup>

Cell line	Passage no.	% FBS in ExCell 401	TCID <sub>50</sub> /ml (± SEM)	% Cells infected (± SEM)
Sf9	92	0	0.008 ± 0.003 × 10 <sup>6</sup>	0.0
HvAM1	289	10	1.96 ± 0.91 × 10 <sup>6</sup>	77.5 ± 0.3
AgOV1	55	10	3.51 ± 1.14 × 10 <sup>6</sup>	74.2 ± 3.0
AgOV2	59	10	24.66 ± 13.65 × 10 <sup>6</sup>	89.9 ± 0.5
AgOV3	75	10	9.68 ± 1.43 × 10 <sup>6</sup>	>99 <sup>b</sup>
HvE1	58	10	14.37 ± 4.37 × 10 <sup>6</sup>	26.7 ± 5.4
HvE2	88	10	62.34 ± 20.19 × 10 <sup>6</sup>	69.8 ± 0.8
HvOV	90	10	51.86 ± 23.13 × 10 <sup>6</sup>	83.5 ± 2.0

<sup>a</sup> Cells (2 × 10<sup>5</sup> cells/ml) were inoculated with virus (1 MOI). Virus was harvested 7 d after infection. Values represent mean titer or percentage, ± SEM. (n = 2). Designations of cell lines are abbreviated. Complete designations are given in Table 1 and "Materials and Methods" section.

<sup>b</sup> Before or on the d of harvest, cell lysis was observed. Therefore, the percent cells producing OBs is an underestimation.

newly generated lines are capable of producing ECV and OBs of a variety of baculoviruses and, in some cases, of producing comparable or higher titers than those reported for other cell lines. Additionally, many of our lines are capable of cell replication and/or baculovirus production in serum-free and/or suspension-culture conditions. These characteristics indicate that our new lines have the potential of being utilized in biopesticide mass production schemes; however, further studies to optimize the production of baculoviruses in these lines are needed, which would include the optimization of their ability to replicate viruses over numerous passages in cell culture.

TABLE 4

PRODUCTION OF PxMNPV BY SELECTED CELL LINES<sup>a</sup>

Cell line	Passage no.	% FBS in ExCell 401	TCID <sub>50</sub> /ml (± SEM)	% Cells infected (± SEM)
Sf9	83	0	6.22 ± 0.60 × 10 <sup>6</sup>	>90 <sup>b</sup>
TnCL1	499	10	82.90 ± 3.60 × 10 <sup>6</sup>	49.9 ± 1.2
AiOV	82	0	7.32 ± 2.68 × 10 <sup>6</sup>	<1 <sup>b</sup>
AiOV	84	10	34.97 ± 3.35 × 10 <sup>6</sup>	5.9 ± 3.7
HvE1	51	0	8.10 ± 1.91 × 10 <sup>6</sup>	<1 <sup>b</sup>
HvE1	55	10	12.85 ± 1.84 × 10 <sup>6</sup>	6.5 ± 2.2
HvE2	80	0	4.73 ± 2.77 × 10 <sup>6</sup>	0.0
HvE2	82	10	25.53 ± 20.88 × 10 <sup>6</sup>	0.0
HvOV	81	0	232.6 ± 54.80 × 10 <sup>6</sup>	28.2 ± 1.9
HvOV	84	10	72.2 ± 10.32 × 10 <sup>6</sup>	28.8 ± 2.5
PxE	55	10	0.65 ± 0.45 × 10 <sup>6</sup>	8.5 ± 1.4
PxLP	67	10	0.04 ± 0.01 × 10 <sup>6</sup>	0
SfTS	63	10	78.77 ± 3.78 × 10 <sup>6</sup>	67.2 ± 5.1
TnE2	58	10	27.67 ± 3.95 × 10 <sup>6</sup>	2.3 ± 0.5
TnTS1	21	10	4.83 ± 1.36 × 10 <sup>6</sup>	28.0 ± 0.5
TnTS3	12	10	11.22 ± 2.12 × 10 <sup>6</sup>	4.2 ± 1.3

<sup>a</sup> Cells (2 × 10<sup>5</sup> cells/ml) were inoculated with virus (1 MOI). Virus was harvested 7 d after infection. Values represent mean titer or percentage, ± SEM (n = 2). Designations of cell lines are abbreviated. Complete designations are given in Table 1 and "Materials and Methods" section.

<sup>b</sup> Percent cells with OB were only estimated for these lines.

## ACKNOWLEDGMENTS

We express our appreciation to Drs. Dwight E. Lynn and Renée M. Wagner for helpful suggestions on the writing of the manuscript, and to Mr. Steve Long for technical assistance. We also wish to gratefully acknowledge the support of American Cyanamid Co. through Cooperative Research Agreement 58-3K95-4-276.

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